

MR 280349



October 26, 2004

8EHQ-1004-159835

CONFIDENTIAL

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Office of Pollution, Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N. W.
Washington, DC 20460
Attention: Section 8(e) Coordinator

Re: **TSCA Section 8(e) Submissions**

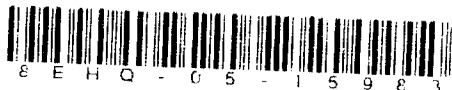
Dear Sir/Madam:

3M Company ("3M") requests that EPA place the attached studies in the TSCA Section 8(e) docket. We have included a master index for these studies identifying the study title, test substance and CAS number. A Confidential Business Information (CBI) version of this index and the studies also is being submitted today pursuant to EPA procedures. 3M has not provided CBI substantiation with this submission, but would be willing to do so at the Agency's request.

3M has concluded that data in these studies may not be, strictly speaking, "corroborative" of previously reported or published information as defined in EPA's reporting guidance or otherwise potentially may warrant 8(e) submission based on EPA's reporting guidance.

3M appreciates EPA's attention to this matter. Please contact the undersigned if you have any questions or require further information regarding this submission.

Very truly yours,



Katherine E. Reed (974)

Dr. Katherine E. Reed, Ph.D
Staff Vice President
Environmental Technology and Safety
Services
(651) 778-4331
kereed@mmm.com

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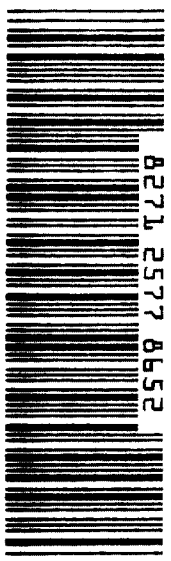
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Matthew Brewer

406

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004
(Confidential Business Information Redacted)

Title	Substance	CAS Information
Aquatic Toxicity Data Sheet: 48hr <i>Daphnia Magna</i>	1,4-dioxane; heptadecafluoro-1-octanesulfonic acid; linear n-ethyl perfluorooctanesulfonamide; n-ethylperfluorooctanesulfonamidoethyl alcohol; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([heptadecafluorooctyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([nonafluorobutyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([pentafluorobutyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([tridecafluorohexyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([undecafluoropentyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; polyethylene glycol; water	1,4-dioxane (123-91-1); heptadecafluoro-1-octanesulfonic acid (1763-23-1); linear n-ethyl perfluorooctanesulfonamide (4151-50-2); n-ethylperfluorooctanesulfonamidoethyl alcohol (1691-99-2); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([heptadecafluorooctyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (29117-08-6); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([nonafluorobutyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (68298-79-3); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([pentafluorobutyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (68298-81-7); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([tridecafluorohexyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (56372-23-7); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([undecafluoropentyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (68298-80-6); polyethylene glycol (25322-68-3); water (7732-18-5)
Multigeneration Daphnid Life Cycle Test	1,4-dioxane; heptadecafluoro-1-octanesulfonic acid; linear n-ethyl perfluorooctanesulfonamide; n-ethylperfluorooctanesulfonamidoethyl alcohol; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([heptadecafluorooctyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([nonafluorobutyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([pentafluorobutyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([tridecafluorohexyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([undecafluoropentyl)sulfonyl]amino[ethyl]-.omega.-hydroxy-; polyethylene glycol; water	1,4-dioxane (123-91-1); heptadecafluoro-1-octanesulfonic acid (1763-23-1); linear n-ethyl perfluorooctanesulfonamide (4151-50-2); n-ethylperfluorooctanesulfonamidoethyl alcohol (1691-99-2); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([heptadecafluorooctyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (29117-08-6); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([nonafluorobutyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (68298-79-3); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([pentafluorobutyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (68298-81-7); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([tridecafluorohexyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (56372-23-7); poly(oxy-1,2-ethanediyl), .alpha.-12-[ethyl]([undecafluoropentyl)sulfonyl]amino[ethyl]-.omega.-hydroxy- (68298-80-6); polyethylene glycol (25322-68-3); water (7732-18-5)
Aquatic Invertebrate Testing - Alkyltins LR 8024-1	Alkyltins: dibutyltin laurate and dibutyltin-di(2 ethylhexoate)	Dibutyltin laurate (CAS 77-58-7); Dibutyltin-di(2 ethylhexoate) (CAS 2781-10-4)
Aquatic Invertebrate Testing - Decosheen Material (LR-8052)	Decosheen Ribbon Materials and pigments; Decosheen Blue in Green Ceres Blue ZV; Decosheen Gold Paste Pigment; Decosheen Royal Blue, Solvent Blue	Decosheen Blue in Green Ceres Blue ZV (CAS 61814-09-3); Decosheen Royal Blue, Solvent Blue (CAS 61814-09-6); Decosheen Gold Paste Pigment (CAS Number 61814-09-5)
R Scratch Remover (Fathead Minnow)	55-65% Water, 20-30% Stoddard Solvent, 1-5% Sodium Silicate, 1-5% Potassium Hydroxide, 0.1-3% Nonylphenoxypoly(oxyethylene)ethanol	Water (CAS 7732-18-5); Stoddard Solvent (CAS 8052-41-3); Sodium Silicate (CAS 1344-09-8); Potassium Hydroxide (CAS 1310-58-3); Nonylphenoxypoly(oxyethylene)ethanol (CAS 9016-45-9)
S Scratch Remover (Fathead Minnow)	60-70% Water, 20-30% Stoddard Solvent, 1-5% Sodium Silicate, 0.1-3% Turgitol NP-33	Water (CAS 7732-18-5); Stoddard Solvent (CAS 8052-41-3); Sodium Silicate (CAS 1344-09-8); Turgitol NP-33 (CAS 9016-45-9)
Octanol Water Partition Coefficient	N-methylperfluorooctane sulfonamidoethanol	CAS 24448-09-7

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004
(Confidential Business Information Redacted)

Title	Substance	CAS Information
CoCl ₂ .6H ₂ O as Co ²⁺ Toxicity to Microtox Reagent	Cobalt (as Co ²⁺ ion) (CoCl ₂ .6H ₂ O)	CAS 7791-13-1
Activated Sludge Respiration Inhibition Test on CoCl ₂ .6H ₂ O as Co ion	Cobalt (as Co ²⁺ ion) (CoCl ₂ .6H ₂ O)	CAS 7791-13-1
Acute Toxicity of CoCl ₂ .6H ₂ O as Co ion to <i>Daphnia magna</i> under Static Exposure Conditions	Cobalt (as Co ²⁺ ion) (CoCl ₂ .6H ₂ O)	CAS 7791-13-1
Acute Toxicity of CoCl ₂ .6H ₂ O as Co ion to Fathead Minnow under Static Exposure Conditions	Cobalt (as Co ²⁺ ion) (CoCl ₂ .6H ₂ O)	CAS 7791-13-1
Freshwater Algae Growth Inhibition Test	Cobalt (as Co ²⁺ ion) (CoCl ₂ .6H ₂ O)	CAS 7791-13-1
<i>Daphnia magna</i> 21-Day Chronic Reproduction Study	N-ethylperfluorooctane sulfonamidoethanol	CAS 1691-99-2
Plant Growth Effects of []	[]	[]
Final Report (<i>Daphnia</i> and Microtox)	Monomethyl ether of hydroquinone	CAS 150-76-5
Microtox Test Results	2-Ethylhexyl Acrylate; Isooctyl Acrylate Monomer; 2-Methylbutyl acrylate; Methyl isoamyl acrylate; Isooctyl Acrylate	2-Ethylhexyl Acrylate (CAS 103-11-7); Isooctyl Acrylate Monomer (CAS 29590-42-9) 2-Methylbutyl acrylate (CAS 44914-03-6); Methyl isoamyl acrylate (CAS 18993-92-1); Isooctyl Acrylate (CAS 29590-42-9)
Phytotoxicity Test Results	[]	[]

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004
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Title	Substance	CAS Information
Isooctyl acrylate: Fish, Acute Toxicity Test	Isooctyl Acrylate Monomer	CAS 29590-42-9
Isooctyl Acrylate: <i>Daphnia</i> sp. Acute Immobilization Test	Isooctyl Acrylate Monomer	CAS 29590-42-9
Isooctyl Acrylate: Alga, Growth Inhibition Test	Isooctyl Acrylate Monomer	CAS 29590-42-9
Isooctyl Acrylate: <i>Daphnia</i> sp. Reproduction Test	Isooctyl Acrylate Monomer	CAS 29590-42-9
Acute Toxicity of [] to the mysid, <i>Mysidopsis bahia</i>	[]	[]
Final Report (Microtox)	[]	[]
Determination of the Partition Coefficient (N _{ow} /O _{cean} /W _{ater}) of T-5896 by High Performance Liquid Chromatography (HPLC)	N-methyl perfluorooctane sulfonamido ethanol; N-methyl perfluorooctane sulfonamidoethyl acrylate	N-methyl perfluorooctane sulfonamido ethanol (CAS 25268-77-3); N-methyl perfluorooctane sulfonamidoethyl acrylate (CAS 24448-09-7)
Activated Sludge Respiration Inhibition Test Results	N-Dodecyltrimethylammonium chloride	CAS = 112-00-5
Final Report (Fish Acute Toxicity)	Miralaine CB (30% Cocamidopropyl betaine = Amides, coco, N-(3-dimethylamino)propyl), alkylation products with chloroacetic acid, sodium salts, 70% Water and Inerts); Miralaine COB (30% Coco/Oleamidopropyl Betaine = 1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivs., inner salt)	Cocamidopropyl betaine (CAS 70851-07-9); Coco/Oleamidopropyl Betaine (CAS 61789-40-0)
A Flow-Through Life-Cycle Toxicity Test With the Saltwater Mysid (<i>Mysidopsis bahia</i>)	Perfluorooctane sulfonate	CAS 1763-23-1
Lithium: Alga, Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
An Early Life-Stage Toxicity Test With the Fathead Minnow (<i>Pimephales promelas</i>)	Perfluorooctane sulfonate	CAS 1763-23-1
Lithium: Fish, Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
Lithium: <i>Daphnia</i> , Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
Summary of Toxicity Testing on OSCI and OSF	Octane sulfonyl chloride and Octane sulfonyl fluoride	Octane sulfonyl fluoride (CAS 7795-95-1), Octane sulfonyl chloride (CAS 4063-63-5)
Toxicity to Microtox Test	Lauryldimethylamineoxide	CAS 1643-20-5

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004
(Confidential Business Information Redacted)

Title	Substance Information	CAS Information
Ecotoxicological Testing of CoCl ₂ ·6H ₂ O as Co ²⁺ ion (Seed Germination and Root Elongation)	Cobalt (as Co ²⁺ ion) (CoCl ₂ ·6H ₂ O)	CAS 7791-13-1

CONFIDENTIAL BUSINESS INFORMATION
SUBJECT TO PROTECTION UNDER THE
TOXIC SUBSTANCES CONTROL ACT
AND OTHER LAWS HAS BEEN
REDACTED FROM THIS DOCUMENT

PFOS:
A FLOW -THROUGH LIFE-CYCLE TOXICITY TEST WITH
THE SALTWATER MYSID (*Mysidopsis bahia*)

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-107
3M LAB REQUEST NO. U2723

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines
OPPTS Number 850.1350

AUTHORS:

Kurt R. Drottar
Henry O. Krueger, Ph.D.

STUDY INITIATION DATE: May 21, 1999

STUDY COMPLETION DATE: April 26, 2000

Submitted to

3M Corporation
Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55144

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

- 2 -

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-107

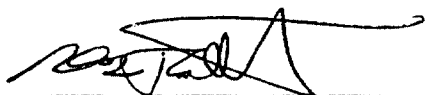
STUDY COMPLETION: April 26, 2000

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984, with the following exceptions:

The test substance was not characterized in accordance with full GLP compliance; however, the characterization was performed according to 3M Standard Operating Procedures and Methods, and all raw data are being maintained in the 3M archives. The test substance is being recharacterized in accordance with GLP.

The stability of the test substance under conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

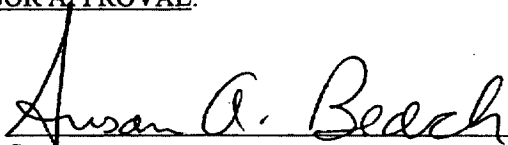


Kurt R. Drott
Senior Biologist

4/26/00

DATE

SPONSOR APPROVAL:



Sponsor

4/27/00

DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO:	
		STUDY DIRECTOR:	MANAGEMENT:
Protocol	May 24, 1999	May 24, 1999	July 16, 1999
Test Substance Preparation	June 10, 1999	June 14, 1999	June 16, 1999
Light Meter Reading and Analytical Sampling	June 16, 1999	June 17, 1999	June 18, 1999
Salinity Measurements	June 17, 1999	June 18, 1999	June 18, 1999
Calibration Standards Preparation	July 21, 1999	July 21, 1999	July 21, 1999
Biological Data and Draft Report	October 1, 4 and 5, 1999	October 6, 1999	October 7, 1999
Analytical Data and Draft Report	October 4 - 7, 1999	October 7, 1999	October 8, 1999
Final Report	April 20, 2000	April 20, 2000	April 24, 2000

Kimberly A. Hoxter
Kimberly A. Hoxter
Quality Assurance Representative

4-24-00
DATE


REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid
(*Mysidopsis bahia*)

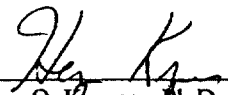
WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-107

STUDY DIRECTOR:


Kurt R. Drott
Senior Biologist

4/26/00
DATE

MANAGEMENT:


Henry O. Krueger, Ph.D.
Director, Aquatic Toxicology and
Non-Target Plants

4/26/00
DATE

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SUMMARY

SPONSOR:	3M Corporation
SPONSOR'S REPRESENTATIVE:	Susan A. Beach
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	454A-107
TEST SUBSTANCE:	PFOS (Perfluorooctane Sulfonic Acid Potassium Salt)
STUDY:	PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (<i>Mysidopsis bahia</i>)
NOMINAL TEST CONCENTRATIONS:	Negative Control, 0.086, 0.17, 0.34, 0.69, 1.4 and 2.7 mg a.i./L
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control, 0.057, 0.12, 0.25, 0.55, 1.3 and 2.6 mg a.i./L
TEST DATES:	Experimental Start (OECD) – May 26, 1999 Experimental Start (EPA) – June 16, 1999 Biological Termination – July 25, 1999 Experimental Termination – July 25, 1999
LENGTH OF FIRST- GENERATION EXPOSURE:	35 Days

TEST ORGANISM:	Saltwater Mysid (<i>Mysidopsis bahia</i>)
SOURCE OF TEST ORGANISMS:	Wildlife International, Ltd. Cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	Juveniles <24 hours old

NOEC:	0.25 mg a.i./L
LOEC:	0.55 mg a.i./L
MATC:	0.37 mg a.i./L

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for 3M Corporation at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The in-life phase of the test was conducted from June 16, 1999 to July 25, 1999. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454A-107 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to evaluate the effects of Perfluorooctane Sulfonic Acid Potassium Salt (PFOS) on the survival, growth and reproduction of the saltwater mysid (*Mysidopsis bahia*) under flow-through test conditions.

EXPERIMENTAL DESIGN

Mysidopsis bahia neonates, less than 24 hours old, were exposed to a geometric series of six test concentrations and a negative (saltwater) control for 35 days. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon known toxicity data. Nominal test concentrations were 0.086, 0.17, 0.34, 0.69, 1.4 and 2.7 mg active ingredient (a.i.)/L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at weekly intervals during the test and at test termination.

Delivery of the test substance was initiated approximately 51 hours prior to the introduction of the neonate mysids to the test water in order to achieve equilibrium of the test substance in the test chambers. Four replicate test chambers, each containing one compartment with 15 mysids, were maintained for each treatment and control group. To begin the test, neonate mysids were impartially distributed in groups of one or two among glass beakers until each beaker contained 15 mysids. The mysids were then transferred to the test compartments. A total of 60 mysids were exposed in each treatment and the control group.

On Day 20 of the test, female and male adults were paired, and the reproduction of the paired mysids was monitored through Day 35. Observations of mortality, clinical signs of toxicity, and reproduction were made

daily. At test termination, the lengths and dry weights of all surviving first-generation mysids were measured. All young produced in the test were removed to a separate test chamber at the same test concentration on a daily basis. The second-generation mysids were exposed for 96 hours under static test conditions.

The no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) were determined by examination of the mortality, growth and reproduction data. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and LOEC.

MATERIALS AND METHODS

The study was conducted based on the procedures outlined in the protocol, "PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)". The protocol was based on procedures outlined in U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines, OPPTS Number 850.1350 (1); and *ASTM Standard E1191-90 Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids* (2).

Test Substance

The test substance was received from 3M Corporation on October 29, 1998 and was assigned Wildlife International, Ltd. identification number 4675. The test substance was described as a white powder. It was identified as [REDACTED] from lot number 217 (T-6295). Information provided by the Sponsor indicated a purity of 90.49%, and an expiration date of 2008. The test substance was stored at ambient room temperature.

Preparation of Test Concentrations

One stock solution was prepared for each of the six concentrations tested. A primary stock was prepared by dissolving the test substance in dilution water at a concentration of 0.0895 mg a.i./mL. The primary stock was stirred with an electric stainless steel top-down mixer for approximately 24 hours to aid in the solubilization of the test substance. After mixing, the primary stock appeared clear and colorless with a foam on the surface. Aliquots of the primary stock solution were proportionally diluted with dilution water to prepare five additional stocks at concentrations of 0.0447, 0.0224, 0.0112, 0.00559 and 0.00280 mg a.i./mL. The six stocks were injected into the diluter mixing chambers (at a rate of 4.60 mL/minute) where they were mixed with dilution water

(at a rate of 150 mL/minute) to achieve the desired test concentrations. All test solutions appeared clear and colorless.

Test Organism

The saltwater mysid, *Mysidopsis bahia* (recently renamed *Americamysis bahia*), was selected as the test species for this study. The saltwater mysid is representative of an important group of aquatic organisms and was selected for use in the test based upon past history of use and ease of culturing in the laboratory. Mysids used in the test were neonates less than 24 hours old and were obtained from cultures maintained by Wildlife International, Ltd., Easton, Maryland.

Adult mysids were held in water from the same source as used during the test. Adult mysids were fed live brine shrimp (*Artemia* sp.) two or three times daily during holding. Brine shrimp were periodically enriched with a fatty acid supplement (ALGAMAC-2000, Aquafauna Bio-Marine, Inc., Hawthorne, California). During the 14-day holding period preceding the test, water temperatures ranged from 25.0 to 25.4°C. The pH of the water ranged from 8.0 to 8.1, salinity ranged from 20 to 23‰ (parts per thousand), and dissolved oxygen ranged from 6.8 to 7.4 mg/L. Instrumentation used for water measurements is described in the *Environmental Conditions* section of this report.

At test initiation, the neonate mysids were carefully collected from the cultures and transferred one and two at a time to glass beakers. Mysids then were transferred from the beakers to the test compartments. All transfers were made using a wide-bore pipette below the surface of the water. The mysids were fed live brine shrimp nauplii three to four times a day during the test to prevent cannibalism (except on the last day of the test).

Test Apparatus

A continuous-flow diluter was used to deliver each concentration of the test substance and a negative (saltwater) control. A peristaltic pump (Cole-Parmer Instrument Company, Chicago, IL) was used to deliver the six test substance stock solutions into mixing chambers assigned to each treatment group. The stock solutions were diluted with dilution water in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters. Rotameters were calibrated prior to test initiation and at weekly intervals thereafter during the test. The flow of test water from each mixing chamber was split and allowed to flow into replicate test chambers. The proportion of test water that was split into each

replicate was checked prior to the test and at weekly intervals thereafter during the test to ensure that flow rates varied by no more than $\pm 10\%$ of the mean for the four replicates.

The diluter was adjusted so that each test chamber received approximately 1 l volume additions of test water every 24 hours. The delivery pump was calibrated before the test and at approximately weekly intervals during the test. The general operation of the diluter was checked visually at least two times per day during the test and once at the end of the test.

Prior to sexual maturity, mysids were held in one compartment placed in each replicate test chamber (15/compartment). The compartments were glass beakers with nylon mesh screen attached to two holes on opposite sides. After mysids attained sexual maturity, reproductive pairs were placed in reproductive compartments (one pair per compartment). The reproductive compartments were glass petri dishes with sides of nylon mesh screen attached with silicone adhesive. The test compartments were placed in 9-L glass aquaria test chambers containing approximately 5 L of test solution. Prior to pairing, the depth of water in a representative test compartment was 6.2 cm. After pairing, the depth of water in a representative test compartment was 5.5 cm. The test chambers were impartially positioned in a temperature-controlled environmental chamber designed to maintain a temperature of $25 \pm 2^\circ\text{C}$. Test compartments were uniquely identified and the test chambers were labeled with the project number, test concentration and replicate. The test chambers for the second generation exposure were 2-L beakers with 1 L of test solution which was dipped out of a test chamber from the appropriate treatment group.

Dilution Water

The water used for culturing and testing was natural seawater collected at Indian River Inlet, Delaware, and was diluted to a salinity of approximately 20‰ with well water. Salinity measurements during the four-week period immediately preceding the test are presented in Appendix I.

The freshly-collected seawater was passed through a sand filter to remove particles greater than approximately 25 μm , and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to delivery to the diluter system, the water again was filtered (0.45 μm) to remove microorganisms and particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in saltwater used by Wildlife International, Ltd. are presented in Appendix II.

Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity ranged from 623 to 815 lux over the surface of the negative control, replicate A test chamber. Light intensity was measured weekly using a SPER Scientific Model 840006C light meter.

Temperature was measured in each test chamber at the beginning and end of the test and at weekly intervals during the test using a liquid-in-glass thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the study was $25 \pm 2^{\circ}\text{C}$. Dissolved oxygen and pH measurements were measured in alternate replicates of each treatment and control group at the beginning and end of the test and at weekly intervals during the test. Salinity was measured daily in alternate replicates of the negative control and the highest treatment group with surviving mysids.

Dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter, and measurements of pH were made using a Fisher Accumet Model 915 pH meter. Salinity was measured using a Bio-Marine, Inc., Aquafauna refractometer.

Biological Observations and Measurements

Observations of the survival and behavior of each first-generation mysid were made daily throughout the test. The criteria for death included lack of movement, absence of respiratory movements, and lack of reaction to gentle prodding. At the time of pairing (Day 20), the sex and maturity of each mysid was determined by microscopic examination, and, when possible, 5 male/female pairs were made for each replicate test chamber. Any immature mysids or extra females were discarded at this time. Sexually mature males, which were left over after pairing were maintained in a separate compartment within that replicate.

After mysids were paired, the number of second-generation mysids were counted and recorded daily until test termination. Second-generation mysids were also observed for abnormal development and aberrant behavior. After each observation, second-generation mysids were collected and exposed at the same test concentration under static test conditions for 96 hours. If a male in a male/female pair died, it was replaced with a male, if

available, from the pool of males maintained in the same replicate. At test termination, the sex of each surviving first-generation mysid was confirmed and the length of each mysid was measured using calipers. Each surviving first-generation was then placed in a drying oven at approximately 60°C for approximately 24 hours. The dry weight of each surviving first-generation mysid was then determined using an analytical balance.

Statistical Analyses

Statistical analyses were performed on survival of the first and second-generation mysids, the number of young released per reproductive day, and the length and dry weight of each surviving first-generation mysid. Survival was evaluated prior to pairing (Day 0 through Day 20) and after pairing (Day 20 through Day 35). Survival data were analyzed using 2 x 2 contingency tables and the Chi-square test to identify treatment groups that were statistically different from the control group.

The analyses of reproduction (number of live young produced per reproductive day) and growth (dry weights and lengths) data included those treatments which did not exhibit a statistical reduction in survival. Analyses included the evaluation of homogeneity of variances using Bartlett's test and the assessment of normality using the Shapiro-Wilk's test. When data were deemed normal and homogeneous, an analysis of variance test was used to determine whether or not statistically significant differences existed among experimental groups. Those treatments statistically different from the control group were identified using Dunnett's test. The results of the statistical analyses were used to aid in the determination of the NOEC and LOEC. The MATC was calculated as the geometric mean of the NOEC and LOEC. All statistical tests were performed using a personal computer with SPSS/PC Version 2.0 (3) or "TOXSTAT Release 3.5" statistical software (4).

Analytical Chemistry

Prior to test initiation, two sets of pretest water samples were collected from two replicate test chambers of both the low and high level treatment groups to determine if nominal concentrations had been achieved. Water samples were also collected from two alternating replicates on Days 0, 7, 14, 21, 28 and 35. Water samples were collected from mid-depth of the test chamber and placed in plastic (Nalgene®) bottles. Samples were analyzed as soon as possible without storage. Analytical procedures used in the analysis of the samples are provided in Appendix III.

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Results of analyses to measure concentrations of PFOS in water samples collected during the test are presented in Table 1 and the analytical chemistry report (Appendix III). Nominal concentrations used in this study were 0.086, 0.17, 0.34, 0.69, 1.4 and 2.7 mg a.i./L. When measured concentrations of samples collected on Days 0, 7, 14, 21, 28 and 35 were averaged, the mean measured concentrations were 0.057, 0.12, 0.25, 0.55, 1.3 and 2.6 mg a.i./L, which represented 66, 71, 74, 80, 93 and 96% of the nominal concentrations, respectively. Mean measured concentrations were used to express the NOEC, LOEC and MATC. Pretest samples collected to verify diluter performance (Appendix III) were not used in the calculation of mean measured concentrations.

Physical and Chemical Measurements of Water

Measurements of salinity in the negative control and the highest treatment group ranged from 19 to 21‰ throughout the test (Table 2). Measurements of pH ranged from 8.2 to 8.4 (Table 3) and temperature was maintained within the 25±2°C range established for the test (Table 4). Dissolved oxygen concentrations remained ≥ 5.8 mg/L (79% of saturation) throughout the test (Table 5).

Survival

A summary of survival from test initiation to pairing on Day 20 is presented in Table 6. In general, all surviving mysids appeared normal. After 20 days of exposure, survival in the negative control group was 78%. Survival in the PFOS treatment groups ≤ 0.55 mg a.i./L ranged from 75 to 92% and were not statistically different from the negative control. Survival in the 1.3 and 2.6 mg a.i./L treatment groups was 32 and 0%, respectively, and was statistically different from the negative control ($p \leq 0.05$).

Observations of survival after pairing (from Day 20 to test termination on Day 35) are presented in Table 7. In general, all surviving mysids appeared normal. Survival in the negative control was 92%. Survival percentages in the PFOS treatment groups ≤ 0.55 mg a.i./L ranged from 90 to 97% and were not statistically different from the negative control. Survival in the 1.3 mg a.i./L treatment group was 57% and was statistically different from the negative control ($p \leq 0.05$).

Reproduction

A summary of the mean number of young produced per reproductive day is presented in Table 8. Young production for individual test compartments is presented in Appendix IV. For each female, the number of reproductive days was defined as the number of days that the female was alive from the day of first brood release of any female in the test to the end of the test. The day of first brood release in this study was Day 22. The mean number of young produced per reproductive day in the negative control groups was 0.315. Reproduction rates in the 0.057, 0.12, 0.25 and 0.55 mg a.i./L treatment groups were 0.261, 0.361, 0.252 and 0.0559 young per reproductive day, respectively. Dunnett's test showed that reproduction was significantly reduced in the 0.55 mg a.i./L treatment group when compared to the negative control ($p \leq 0.05$). The 1.3 and 2.6 mg a.i./L treatment groups were not included in the statistical analysis of the reproduction data due to a statistically significant difference in survival.

Growth

Summaries of the lengths and dry weights of the surviving adult mysids are presented in Tables 9 and 10, respectively. Individual measurements are provided in Appendices V and VI. The mean length and mean dry weight in the negative control group were 6.43 mm and 0.63 mg, respectively. Mysids exposed to PFOS at concentrations ≤ 0.25 mg a.i./L showed no statistically significant reductions in length or dry weight ($p > 0.05$). Mysids exposed to 0.55 mg a.i./L showed statistically significant reductions in both length and dry weight ($p \leq 0.05$). The 1.3 and 2.6 mg a.i./L treatment groups were not included in the statistical analyses of growth due to a statistically significant difference in survival.

Second Generation Acute Exposure

The results of the second generation exposure are presented in Table 11. After 96-hours, control survival was 96%. Survival in all PFOS treatment groups was $\geq 95\%$ and was not statistically different from the controls. All surviving mysids in the second generation exposure appeared normal with no overt signs of toxicity.

CONCLUSIONS

There were no statistically significant effects on survival, reproduction or growth of mysid shrimp (*Mysidopsis bahia*) exposed to PFOS at concentrations of ≤ 0.25 mg a.i./L for 35 days. Reproduction, length and

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dry weight were the most sensitive biological endpoints in this study. Mysid shrimp exposed to 1.3 and 2.6 mg a.i./L had significantly reduced survival in comparison to the negative control. Mysids exposed to 0.55 mg a.i./L had significantly reduced reproduction, length and dry weight in comparison to the negative control. Consequently, the LOEC, based on reproduction, length and dry weight, was 0.55 mg a.i./L. The NOEC was 0.25 mg a.i./L and the MATC was calculated to be 0.37 mg a.i./L. Second generation mysids exposed to PFOS during a static 96-hour exposure showed no adverse effects.

REFERENCES

- 1 **U.S. Environmental Protection Agency.** 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.1350: *Mysid Chronic Toxicity Test*.
- 2 **ASTM Standard E1191-90.** 1991. *Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids*, American Society for Testing and Materials.
- 3 **SPSS Inc.** 1988. SPSS/PC+ Version 2.0. Chicago, Illinois.
- 4 **West, Inc. and D. D. Gulley.** 1996. TOXSTAT Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

Table I

Summary of Analytical Chemistry Data

Sponsor: 3M Corporation Test Substance: PFOS Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i> Dilution Water: Filtered Saltwater											
Nominal Test Concentration (mg a.i./L)	Measured Concentration (mg a.i./L)							Mean (mg a.i./L)	Percent of Nominal		
	Day 0 ¹	Day 7 ²	Day 14 ¹	Day 21 ²	Day 28 ¹	Day 35 ²					
Negative Control	<LOQ ³	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		-	-		
0.086	0.0694 0.0578	0.0478 0.0619	0.0606 0.0614	0.0554 0.0509	0.0515 0.0569	0.0580 0.0514		0.057	66		
0.17	0.125 0.114	0.0778 0.125	0.124 0.127	0.0970 0.112	0.122 0.128	0.124 0.119		0.12	71		
0.34	0.289 0.286	0.231 0.197	0.276 0.253	0.227 0.212	0.262 0.271	0.278 0.251		0.25	74		
0.69	0.562 0.659	0.581 0.450	0.543 0.542	0.516 0.528	0.529 0.544	0.556 0.583		0.55	80		
1.4	1.23 1.32	1.13 1.20	1.35 1.27	1.23 1.15	1.39 1.39	1.26 1.20		1.3	93		
2.7	2.56 2.79	2.58 2.30	2.54 2.69	⁴ ⁴	- -	- -		2.6	96		

¹ Replicates A and C measured.² Replicates B and D measured.³ LOQ - Limit of quantitation was 0.0458 mg a.i./L⁴ Samples not collected due to 100% mortality.

Table 2
Salinity of Water in the Negative Control and Highest Treatment Group Test Chambers

Sponsor:		3M Corporation	
Test Substance:		PFOS	
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>	
Dilution Water:		Filtered Saltwater	
Day	Replicate	Negative Control Salinity (‰)	2.6 mg a.i./L / 1.3 mg a.i./L Salinity (‰)
0	A	20	20
1	B	21	21
2	C	20	20
3	D	20	19
4	A	20	20
5	B	20	20
6	C	20	20
7	D	20	20
8	A	20	20
9	B	20	20
10	C	20	20
11	D	20	20
12	A	20	20
13	B	20	20
14	C	21	20
15 ¹	D	20	20
16	A	20	20
17	B	20	20
18	C	20	20
19	D	20	20
20	A	20	20
21	B	20	20
22	C	20	20
23	D	20	20
24	A	20	20
25	B	20	20
26	C	20	20
27	D	20	20
28	A	20	20
29	B	20	20
30	C	20	20
31	D	20	20
32	A	20	20
33	B	20	20
34	C	20	20
35	D	20	20

¹ On Days 15-35, salinity was measured in the 1.3 mg a.i./L treatment group due to 100% mortality in the 2.6 mg a.i./L treatment group.

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Table 3

pH of Water in the Test Chambers

Sponsor:		3M Corporation					
Test Substance:		PFOS					
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>					
Dilution Water:		Filtered Saltwater					
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Day					
		0	7	14	21	28	35
Negative Control	A	8.4	--	--	--	8.3	--
	B	--	8.2	--	--	--	8.3
	C	--	--	8.3	--	--	--
	D	--	--	--	8.3	--	--
0.057	A	8.4	--	--	--	8.3	--
	B	--	8.2	--	--	--	8.3
	C	--	--	8.3	--	--	--
	D	--	--	--	8.3	--	--
0.12	A	8.4	--	--	--	8.3	--
	B	--	8.2	--	--	--	8.3
	C	--	--	8.3	--	--	--
	D	--	--	--	8.3	--	--
0.25	A	8.4	--	--	--	8.3	--
	B	--	8.2	--	--	--	8.3
	C	--	--	8.3	--	--	--
	D	--	--	--	8.3	--	--
0.55	A	8.4	--	--	--	8.3	--
	B	--	8.2	--	--	--	8.3
	C	--	--	8.3	--	--	--
	D	--	--	--	8.3	--	--
1.3	A	8.4	--	--	--	8.3	--
	B	--	8.3	--	--	--	8.3
	C	--	--	8.3	--	--	--
	D	--	--	--	8.3	--	--
2.6	A	8.4	--	--	--	--	--
	B	--	8.3	--	--	--	--
	C	--	--	8.3	--	--	--
	D	--	--	--	-- ¹	--	--

¹Measurements discontinued due to 100% mortality.

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Table 4

Temperature (°C) of Water in the Test Chambers

Sponsor:		3M Corporation					
Test Substance:		PFOS					
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>					
Dilution Water:		Filtered Saltwater					
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Day					
		0	7	14	21	28	35
Negative Control	A ¹	25.0	25.2	24.8	24.9	25.2	25.0
	B	25.0	25.1	24.8	24.9	25.2	24.9
	C	25.0	25.0	24.6	24.9	25.2	25.0
	D	25.0	25.1	24.5	24.9	25.1	25.0
0.057	A	25.0	25.2	24.8	25.0	25.1	24.9
	B	25.0	25.3	25.0	25.0	25.1	25.0
	C	25.0	25.2	24.9	25.0	25.1	25.1
	D	25.0	25.2	25.0	25.0	25.2	25.0
0.12	A	25.0	25.1	24.8	25.1	25.1	24.9
	B	24.9	25.1	24.9	25.1	25.0	24.9
	C	25.0	25.2	24.9	25.1	25.0	24.9
	D	25.0	25.2	24.9	25.0	25.1	24.9
0.25	A	24.8	25.1	24.7	25.1	25.0	25.0
	B	24.8	25.0	24.9	25.1	25.1	25.0
	C	24.9	25.0	24.8	25.1	25.0	25.0
	D	24.9	25.0	24.9	25.1	25.1	24.9
0.55	A	24.9	25.0	24.9	25.0	25.1	25.0
	B	24.7	25.0	24.8	25.0	25.0	24.9
	C	24.8	25.0	24.8	25.0	25.0	24.9
	D	24.7	25.0	24.8	25.0	25.0	24.9
1.3	A	24.6	24.8	24.4	24.5	24.8	24.8
	B	24.7	24.9	24.7	24.5	24.8	24.8
	C	24.9	25.0	24.5	24.7	24.8	24.9
	D	25.1	25.1	25.1	25.0	25.0	24.9
2.6	A	24.9	25.1	25.0	-- ²	--	--
	B	24.8	25.0	-- ²	--	--	--
	C	24.8	25.0	25.1	-- ²	--	--
	D	24.8	25.1	25.1	-- ²	--	--

¹ Temperature measured continuously during the test ranged from 24.5 to 25.5°C.² Measurements discontinued due to 100% mortality.

Table 5

Dissolved Oxygen Content (mg/L) of Water in the Test Chambers¹

Sponsor:		3M Corporation					
Test Substance:		PFOS					
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>					
Dilution Water:		Filtered Saltwater					
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Day					
		0	7	14	21	28	35
Negative Control	A	6.1	--	--	--	6.0	--
	B	--	6.4	--	--	--	6.3
	C	--	--	6.2	--	--	--
	D	--	--	--	6.0	--	--
0.057	A	6.1	--	--	--	6.0	--
	B	--	6.3	--	--	--	6.2
	C	--	--	6.0	--	--	--
	D	--	--	--	6.0	--	--
0.12	A	6.1	--	--	--	6.0	--
	B	--	6.1	--	--	--	6.3
	C	--	--	6.3	--	--	--
	D	--	--	--	6.0	--	--
0.25	A	6.1	--	--	--	5.8	--
	B	--	6.1	--	--	--	6.3
	C	--	--	6.2	--	--	--
	D	--	--	--	5.8	--	--
0.55	A	6.1	--	--	--	6.0	--
	B	--	6.2	--	--	--	6.2
	C	--	--	6.2	--	--	--
	D	--	--	--	6.0	--	--
1.3	A	6.1	--	--	--	6.0	--
	B	--	6.2	--	--	--	6.3
	C	--	--	6.2	--	--	--
	D	--	--	--	5.9	--	--
2.6	A	6.1	--	--	--	--	--
	B	--	6.2	--	--	--	--
	C	--	--	6.3	--	--	--
	D	--	--	--	-- ²	--	--

¹ A dissolved oxygen concentration of 4.4 mg/L represents 60% saturation at 25°C in saltwater with a salinity of 20‰.² Measurements discontinued due to 100% mortality.

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Table 6

Survival of Juvenile Mysids Exposed to PFOS
(Day 0 Through Pairing on Day 20)

Sponsor:	3M Corporation			
Test Substance:	PFOS			
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water:	Filtered Saltwater			
Mean Measured Test Concentration (mg a.i./L)	Replicate	Survival on Day 20 (Pairing)		
		No. Alive/No. Exposed	Total	Percent Survival
Negative Control	A	14/15	47/60	78
	B	10/15		
	C	14/15		
	D	9/15		
0.057	A	14/15	55/60	92
	B	13/15		
	C	15/15		
	D	13/15		
0.12	A	14/15	45/60	75
	B	10/15		
	C	9/15		
	D	12/15		
0.25	A	13/15	49/60	82
	B	10/15		
	C	14/15		
	D	12/15		
0.55	A	11/15	50/60	83
	B	13/15		
	C	13/15		
	D	13/15		
1.3	A	7/15	19/60	32*
	B	4/15		
	C	4/15		
	D	4/15		
2.6	A	0/15	0/60	0*
	B	0/15		
	C	0/15		
	D	0/15		
* Indicates a significant difference from the negative control using 2 X 2 contingency tables ($p \leq 0.05$).				

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Table 7

Survival of Adult Mysids Exposed to PFOS
(Day 20 Through Test Termination on Day 35)

Sponsor:	3M Corporation			
Test Substance:	PFOS			
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water:	Filtered Saltwater			
Mean Measured Test Concentration (mg a.i./L)	Replicate	Survival at Test Termination		
		No. Alive/No. Exposed	Total	Percent
Negative Control	A	11/12	36/39	92
	B	7/8		
	C	10/11		
	D	8/8		
0.057	A	12/13	44/46	96
	B	12/12		
	C	10/11		
	D	10/10		
0.12	A	12/12	36/40	90
	B	7/9		
	C	7/9		
	D	10/10		
0.25	A	10/10	36/37	97
	B	8/8		
	C	9/10		
	D	9/9		
0.55	A	7/8	35/37	95
	B	10/10		
	C	10/10		
	D	8/9		
1.3	A	3/7	8/14	57*
	B	4/4		
	C	0/0		
	D	1/3		

*Indicates a significant difference from the negative control using a 2 X 2 contingency table ($p \leq 0.05$).

Table 8

Mean Number of Young Produced Per Reproductive Day

Sponsor: 3M Corporation					
Test Substance: PFOS					
Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i>					
Dilution Water: Filtered Saltwater					
Mean Measured Test Concentration (mg a.i./L)	Replicate	Number of Reproductive Days	Number of Young Produced	Mean Number of Young/Reproductive Day	Overall Mean \pm s
Negative Control	A	70	18	0.257	0.315 \pm 0.0925
	B	53	14	0.264	
	C	70	20	0.286	
	D	42	19	0.452	
0.057	A	60	17	0.283	0.261 \pm 0.0873
	B	70	14	0.200	
	C	70	13	0.186	
	D	56	21	0.375	
0.12	A	70	21	0.300	0.361 \pm 0.101
	B	46	22	0.478	
	C	54	22	0.407	
	D	70	18	0.257	
0.25	A	70	19	0.271	0.252 \pm 0.0723
	B	56	12	0.214	
	C	61	21	0.344	
	D	56	10	0.179	
0.55	A	54	3	0.0556	0.0559* \pm 0.0376
	B	56	6	0.107	
	C	70	3	0.0429	
	D	56	1	0.0179	
1.3 ¹	A	22	0	0.000	0.000 \pm 0.000
	B	14	0	0.000	
	C	0	0	0.000	
	D	11	0	0.000	

This treatment group was not included in the statistical analyses of reproduction due to a statistically significant difference in survival.

Indicates a significant difference from the negative control using Dunnett's test ($p \leq 0.05$).

Table 9

Mean Total Length of Adult Mysids at the End of the 35-Day Test Period

Sponsor:	3M Corporation		
Test Substance:	PFOS		
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>		
Dilution Water:	Filtered Saltwater		
Mean Measured Concentration (mg a.i./L)	Replicate	Replicate Mean (mm)	Overall Mean \pm s (mm)
Negative Control	A	6.45	6.43 \pm 0.0634
	B	6.34	
	C	6.46	
	D	6.48	
0.057	A	6.38	6.43 \pm 0.0729
	B	6.45	
	C	6.52	
	D	6.36	
0.12	A	6.55	6.56 \pm 0.105
	B	6.65	
	C	6.62	
	D	6.42	
0.25	A	6.48	6.40 \pm 0.0548
	B	6.38	
	C	6.36	
	D	6.38	
0.55	A	6.05	6.14* \pm 0.0794
	B	6.11	
	C	6.16	
	D	6.24	
1.3 ¹	A	5.93	5.85 \pm 0.178
	B	5.98	
	C	--	
	D	5.65	

¹ This treatment group was not included in the statistical analyses of total length due to a statistically significant difference in survival.

* Indicates a significant difference from the negative control using Dunnett's test ($p \leq 0.05$).

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Table 10

Mean Dry Weight of Adult Mysids at the End of the 35-Day Test Period

Sponsor: 3M Corporation			
Test Substance: PFOS			
Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water: Filtered Saltwater			
Mean Measured Concentration (mg a.i./L)	Replicate	Replicate Mean (mg)	Overall Mean \pm s (mg)
Negative Control	A	0.599	0.634 \pm 0.0510
	B	0.706	
	C	0.596	
	D	0.636	
0.057	A	0.616	0.599 \pm 0.0276
	B	0.627	
	C	0.590	
	D	0.565	
0.12	A	0.647	0.641 \pm 0.0241
	B	0.664	
	C	0.644	
	D	0.607	
0.25	A	0.644	0.622 \pm 0.0227
	B	0.601	
	C	0.603	
	D	0.639	
0.55	A	0.556	0.562* \pm 0.00624
	B	0.558	
	C	0.563	
	D	0.570	
1.3 ¹	A	0.440	0.436 \pm 0.0441
	B	0.478	
	C	--	
	D	0.390	

¹ This treatment group was not included in the statistical analyses of dry weight due to a statistically significant difference in survival.

* Indicates a significant difference from the negative control using Dunnett's test ($p \leq 0.05$).

Table 11

Survival of Second-Generation Mysids Exposed to PFOS
During 96-Hour Static Exposures

Sponsor:	3M Corporation		
Test Substance:	PFOS		
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>		
Dilution Water:	Filtered Saltwater		
Mean Measured Test Concentration (mg a.i./L)	Total No. Exposed	No. Alive After 96 Hours of Exposure (Observations ¹)	Percent Survival
Negative Control	71	68 (AN)	96
0.057	65	63 (AN)	97
0.12	83	79 (AN)	95
0.25	62	59 (AN)	95
0.55	13	13 (AN)	100
¹ AN = Appears Normal.			

APPENDIX I

Dilution Water Salinity Measured During the
4-Week Period Immediately Preceding the Test

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>
Dilution Water:	Filtered Saltwater

	Mean	Range
Salinity (‰)	20 (N = 4)	20 - 20

APPENDIX II
Analyses of Pesticides, Organics, Metals and Other Inorganics
in Wildlife International, Ltd. Saltwater¹

ANALYSIS		MEASURED CONCENTRATION	
Miscellaneous Measurements			
Total Dissolved Solids		23,500	mg/L
Ammonia Nitrogen	<	0.050	mg/L
Total Organic Carbon ²	<	1.0	mg/L
Total Cyanide	<	10.0	µg/L
Organochlorines and PCBs			
Aldrin	<	0.005	µg/L
Alpha BHC	<	0.005	µg/L
Beta BHC	<	0.005	µg/L
Delta BHC	<	0.005	µg/L
Gamma BHC (Lindane)	<	0.006	µg/L
Chlordane	<	0.025	µg/L
DDD, pp'	<	0.006	µg/L
DDE, pp'	<	0.005	µg/L
DDT, pp'	<	0.008	µg/L
Dieldrin	<	0.005	µg/L
Endosulfan, A	<	0.005	µg/L
Endosulfan, B	<	0.005	µg/L
Endosulfan Sulfate	<	0.018	µg/L
Endrin	<	0.010	µg/L
Endrin Aldehyde	<	0.005	µg/L
Heptachlor	<	0.005	µg/L
Methoxychlor	<	0.007	µg/L
Heptachlor Epoxide	<	0.005	µg/L
Toxaphene	<	0.500	µg/L
PCB-1016	<	0.260	µg/L
PCB-1221	<	0.260	µg/L
PCB-1232	<	0.260	µg/L
PCB-1242	<	0.720	µg/L
PCB-1248	<	0.720	µg/L
PCB-1254	<	0.720	µg/L
PCB-1260	<	0.720	µg/L
Metals and Other Inorganics			
Aluminum ³	<	100	µg/L
Arsenic ³	<	25.0	µg/L
Beryllium ³	<	0.50	µg/L
Cadmium ³	<	1.0	µg/L
Calcium ³		235	mg/L
Chromium ³	<	2.0	µg/L
Cobalt ³	<	1.0	µg/L
Copper ³	<	20.0	µg/L
Iron ³	<	100	µg/L
Lead ³	<	10.0	µg/L
Magnesium ³		760	mg/L
Manganese ³	<	4.0	µg/L
Mercury ³	<	0.20	µg/L
Molybdenum ³	<	2.0	µg/L
Nickel ³	<	20.0	µg/L
Potassium ³		277	mg/L
Selenium ³	<	25.0	µg/L
Silver ³	<	1.0	µg/L
Sodium ³		6,010	mg/L
Zinc ³	<	20.0	µg/L

¹ Analyses performed by QST Environmental, Gainesville, Florida for samples collected on November 3 through November 7, 1997.

² Analyses performed by Wildlife International, Ltd. for the sample collected on November 5, 1997.

³ Analyses performed by Wildlife International, Ltd. for samples collected on November 5 through 7, 1997.

APPENDIX III

THE ANALYSES OF PFOS IN SALTWATER
IN SUPPORT OF
WILDLIFE INTERNATIONAL PROJECT NO.: 454A-107


REPORT APPROVAL

SPONSOR: 3M Corporation

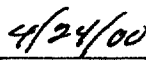
TITLE: PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid
(*Mysidopsis bahia*)

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454A-107

PRINCIPAL INVESTIGATOR:




Jon A. MacGregor, B.S.
Scientist




DATE

MANAGEMENT:



Willard B. Nixon, Ph.D.
Manager, Analytical Chemistry



DATE

Introduction

Saltwater samples were collected from a flow-through life-cycle toxicity study designed to determine the effects of PFOS (Perfluorooctane Sulfonic Acid Potassium Salt) to the saltwater mysid (*Mysidopsis bahia*). This study was conducted by Wildlife International Ltd. and identified as Project No.: 454A-107. The analyses of these water samples were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Samples were received for analysis between June 15, 1999 and July 21, 1999 and were analyzed between June 15, 1999 and July 22, 1999.

Test Substance and Internal Standard

The test substance used for this study was Wildlife International Ltd. identification number 4675. The test substance was used to prepare calibration and matrix fortification samples.

The internal standard was received from 3M Corporation on July 2, 1998 and was assigned Wildlife International Ltd. identification number 4526 upon receipt. The internal standard, a granular material, was identified as: 1H, 1H, 2H, 2H Perfluorooctane Sulfonic Acid, Chemical Abstract Number: 27619-97-2. The standard was stored under ambient conditions.

Analytical Method

The method used for the analysis of the saltwater samples was developed at Wildlife International Ltd. and entitled "Analytical Method for the Determination of PFOS in Freshwater, Saltwater, and Algal Medium". This methodology was included as Appendix II of Wildlife International Ltd. protocol number 454/011299/MVAL/SUB454. It was based upon methodology provided by 3M Corporation.

Samples were diluted in a 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v) so that they fell within the calibration range of the PFOS methodology.

Concentrations of the PFOS in the standards and samples were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer API 100LC Mass Spectrometer equipped with a Perkin-

Elmer TurbolonSpray ion source. HPLC separations were achieved using a Keystone Betasil C₁₈ analytical column (50 mm x 2 mm I.D., 3 µm particle size). The instrument parameters are summarized in Table 1. A method flowchart is provided in Figure 1.

Calibration Curve and Limit of Quantitation

Calibration standards of PFOS prepared in a 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v), ranging in concentration from 0.000915 to 0.00915 mg a.i./L, were analyzed with the samples. The same and most prominent peak response for PFOS was utilized to monitor PFOS in all calibration, quality control, and study samples. No attempt was made to quantify PFOS on the basis of individual isomeric components. Linear regression equations were generated using peak area response ratios (PFOS : internal standard) versus the respective concentration ratios (PFOS: internal standard) of the calibration standards. A typical calibration curve is presented in Figure 2. The concentration of PFOS in the samples was determined by substituting the peak area response ratios into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards are presented in Figures 3 and 4, respectively.

The method limit of quantitation (LOQ) for these analyses was set at 0.0458 mg a.i./L calculated as the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Matrix Blank and Fortification Samples

Six matrix blank samples were analyzed to determine possible interference. No interferences were observed at or above the LOQ during samples analyses (Table 2). A representative ion chromatogram of a matrix blank is presented in Figure 5.

Saltwater was fortified at 0.0823, 0.366 and 3.66 mg a.i./L and analyzed concurrently with the samples to determine the mean procedural recovery (Table 3). Sample concentrations were not corrected for the mean procedural recovery of 92.8%. A representative ion chromatogram of a matrix fortification is presented in Figure 6.

Example Calculations

Sample number 454A-107-3, nominal concentration of 0.086 mg a.i./L in saltwater.

Peak Area Ratio = Analyte Peak Area/Internal Standard Peak Area

Concentration Ratio = Concentration of Analyte/Concentration of Internal Standard

Internal Standard Concentration: 0.100 mg/L

Initial Volume: 0.500 mL

Final Volume: 25.0 mL

Dilution Factor: 50.0

PFOS Peak Area: 12963

Internal Standard Peak Area: 145150

Peak Area Ratio: 0.08931

Calibration curve equation.

Slope: 5.76026

Intercept: 0.00940

Curve is weighted (1/x).

$$\text{PFOS (mg a.i./L) at instrument} = \frac{\text{Peak area ratio} - (\text{Y-intercept})}{\text{Slope}} \times \text{Internal Standard Concentration}$$

$$= \frac{0.08931 - 0.00940}{5.76026} \times 0.100$$

$$= 0.001387$$

$$\text{PFOS (mg a.i./L) in sample} = \text{PFOS (mg a.i./L) at instrument} \times \text{Dilution Factor}$$

$$= 0.001387 \times 50.0$$

$$= 0.06935$$

$$\text{Percent of Nominal Concentration} = \frac{\text{PFOS (mg a.i./L) in sample}}{\text{PFOS (mg a.i./L) nominal}} \times 100$$

$$= \frac{0.06935}{0.086} \times 100 = 80.6\%$$

RESULTS

Sample Analysis

Saltwater samples were collected from the flow-through life-cycle toxicity study with the saltwater mysid (*Mysidopsis bahia*) at pre-test, June 15, 1999, at test initiation, June 16, 1999 (Day 0), and weekly during the test through test termination on July 21, 1999 (Day 35). The measured concentrations of PFOS in the samples collected at pre-test ranged from 57.3 to 99.3% of the nominal concentrations (Table 4). The measured concentrations of PFOS in the samples collected at initiation of exposure of the test organisms (Hour 0) ranged from 67.1 to 103% of the nominal concentrations (Table 5). Samples collected at Day 7, Day 14, Day 21 and Day 28 had measured concentration ranges of 45.8 to 95.6%, 70.5 to 99.6%, 57.1 to 87.9% and 59.9 to 99.3% of nominal values, respectively. Samples collected at test termination (Day 35) had a measured concentration range of 59.8 to 90.0% of nominal values. A representative ion chromatogram of a test sample is shown in Figure 7.

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Table 1

Typical HPLC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 100LC Mass Spectrometer equipped with a Perkin-Elmer TurbolonSpray ion source. Operated in selective ion monitoring mode (SIM).
ANALYTICAL COLUMN:	Keystone Betasil C ₁₈ column (50 mm x 2 mm I.D., 3 µm particle size)
OVEN TEMPERATURE:	30°C
STOP TIME:	5.00 minutes
FLOW RATE:	0.220 mL/minute
MOBILE PHASE:	72.0% Methanol : 28.0% NANOpure® Water containing 0.1% Formic Acid
INJECTION VOLUME:	25.0 µL
PFOS RETENTION TIME:	Approximately 3.8 to 4.5 minutes
INTERNAL STANDARD RETENTION TIME:	Approximately 2.6 to 3.0 minutes
PFOS MONITORED MASS:	498.6 amu
INTERNAL STANDARD MONITORED MASS:	426.7 amu

Table 2

Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of
Number (454A-107-)	Type	PFOS ¹ (mg a.i./L)
MAB-1	Matrix Blank	< LOQ
MAB-2	Matrix Blank	< LOQ
MAB-3	Matrix Blank	< LOQ
MAB-4	Matrix Blank	< LOQ
MAB-5	Matrix Blank	< LOQ
MAB-6	Matrix Blank	< LOQ

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (454A-107-)	Concentrations of PFOS (mg a.i./L)		Percent Recovered
	Fortified	Measured	
MAS-1	0.0823	0.0748	90.9
MAS-4	0.0823	0.0828	101
MAS-7	0.0823	0.0673	81.8
MAS-10	0.0823	0.0662	80.4
MAS-13	0.0823	0.0693	84.2
MAS-16	0.0823	0.0787	95.6
MAS-2	0.366	0.350	95.6
MAS-5	0.366	0.359	98.1
MAS-8	0.366	0.314	85.8
MAS-11	0.366	0.303	82.8
MAS-14	0.366	0.360	98.4
MAS-17	0.366	0.287	78.4
MAS-3	3.66	3.29	89.9
MAS-6	3.66	3.54	96.7
MAS-9	3.66	3.39	92.6
MAS-12	3.66	3.88	106
MAS-15	3.66	3.80	104
MAS-18	3.66	3.98	109
Mean = 92.8			
Standard Deviation = 9.19			
CV = 9.90			
N = 18			

Table 4

Measured Concentrations of PFOS in Pre-test Diluter Verification Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Hours)	PFOS Measured Concentration (mg a.i./L) ^{1,2}	Percent of Nominal
0.086	PT-1	-24	0.0581	67.6
	PT-2	-24	0.0493	57.3
	PT-5	-24	0.0627	72.9
	PT-6	-24	0.0606	70.5
2.7				--
	PT-3	-24	2.21	81.9
	PT-4	-24	2.34	86.7
	PT-7	-24	2.56	94.8
	PT-8	-24	2.68	99.3

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

² Measured values of matrix blanks and matrix fortification samples analyzed concurrently with pre-test samples were <LOQ, 83.0, 95.6, 100, <LOQ, 93.4, 85.0 and 106 percent of nominal concentrations for pre-test 1 and pre-test 2, respectively.

Table 5

Measured Concentrations of PFOS in Saltwater Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Day)	PFOS Measured Concentration (mg a.i./L) ¹	Percent of Nominal
0.0	1	0	< LOQ	--
	2	0	< LOQ	--
	15	7	< LOQ	--
	16	7	< LOQ	--
	29	14	< LOQ	--
	30	14	< LOQ	--
	43	21	< LOQ	--
	44	21	< LOQ	--
	55	28	< LOQ	--
	56	28	< LOQ	--
	67	35	< LOQ	--
	68	35	< LOQ	--
0.086	3	0	0.0694	80.7
	4	0	0.0578	67.2
	17	7	0.0478	55.6
	18	7	0.0619	72.0
	31	14	0.0606	70.5
	32	14	0.0614	71.4
	45	21	0.0554	64.4
	46	21	0.0509	59.2
	57	28	0.0515	59.9
	58	28	0.0569	66.2
	69	35	0.0580	67.4
	70	35	0.0514	59.8

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Table 5 (continued)

Measured Concentrations of PFOS in Saltwater Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Day)	PFOS Measured Concentration (mg a.i./L) ¹	Percent of Nominal
0.17	5	0	0.125	73.5
	6	0	0.114	67.1
	19	7	0.0778	45.8
	20	7	0.125	73.5
	33	14	0.124	72.9
	34	14	0.127	74.7
	47	21	0.0970	57.1
	48	21	0.112	65.9
	59	28	0.122	71.8
	60	28	0.128	75.3
	71	35	0.124	72.9
	72	35	0.119	70.0
0.34	7	0	0.289	85.0
	8	0	0.286	84.1
	21	7	0.231	67.9
	22	7	0.197	57.9
	35	14	0.276	81.2
	36	14	0.253	74.4
	49	21	0.227	66.8
	50	21	0.212	62.4
	61	28	0.262	77.1
	62	28	0.271	79.7
	73	35	0.278	81.8
	74	35	0.251	73.8

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Table 5 (continued)

Measured Concentrations of PFOS in Saltwater Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Day)	PFOS Measured Concentration (mg a.i./L) ¹	Percent of Nominal
0.69	9	0	0.562	81.4
	10	0	0.659	95.5
	23	7	0.581	84.2
	24	7	0.450	65.2
	37	14	0.543	78.7
	38	14	0.542	78.6
	51	21	0.516	74.8
	52	21	0.528	76.5
	63	28	0.529	76.7
	64	28	0.544	78.8
	75	35	0.556	80.6
	76	35	0.583	84.5
1.4	11	0	1.23	87.9
	12	0	1.32	94.3
	25	7	1.13	80.7
	26	7	1.20	85.7
	39	14	1.35	96.4
	40	14	1.27	90.7
	53	21	1.23	87.9
	54	21	1.15	82.1
	65	28	1.39	99.3
	66	28	1.39	99.3
	77	35	1.26	90.0
	78	35	1.20	85.7

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Table 5 (continued)

Measured Concentrations of PFOS in Saltwater Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Day)	PFOS Measured Concentration (mg a.i./L) ¹	Percent of Nominal
2.7	13	0	2.56	94.8
	14	0	2.79	103
	27	7	2.58	95.6
	28	7	2.30	85.2
	41	14	2.54	94.1
	42	14	2.69	99.6

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

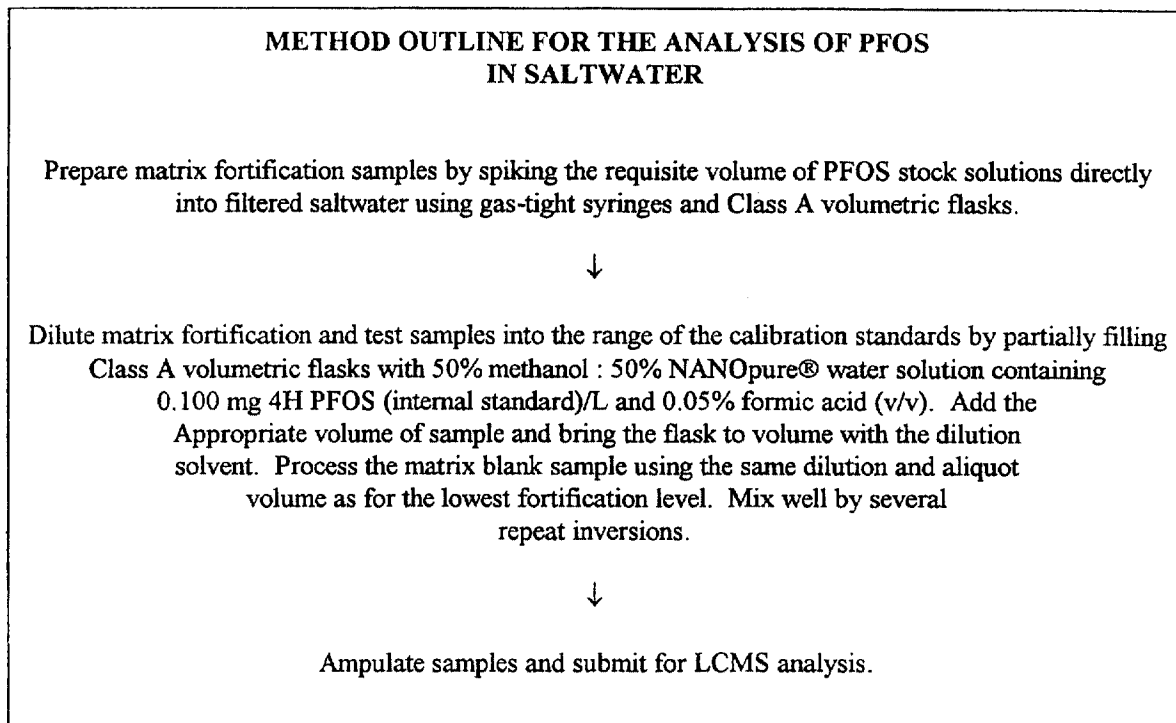


Figure 1. Analytical method flowchart for the analysis of PFOS in saltwater.

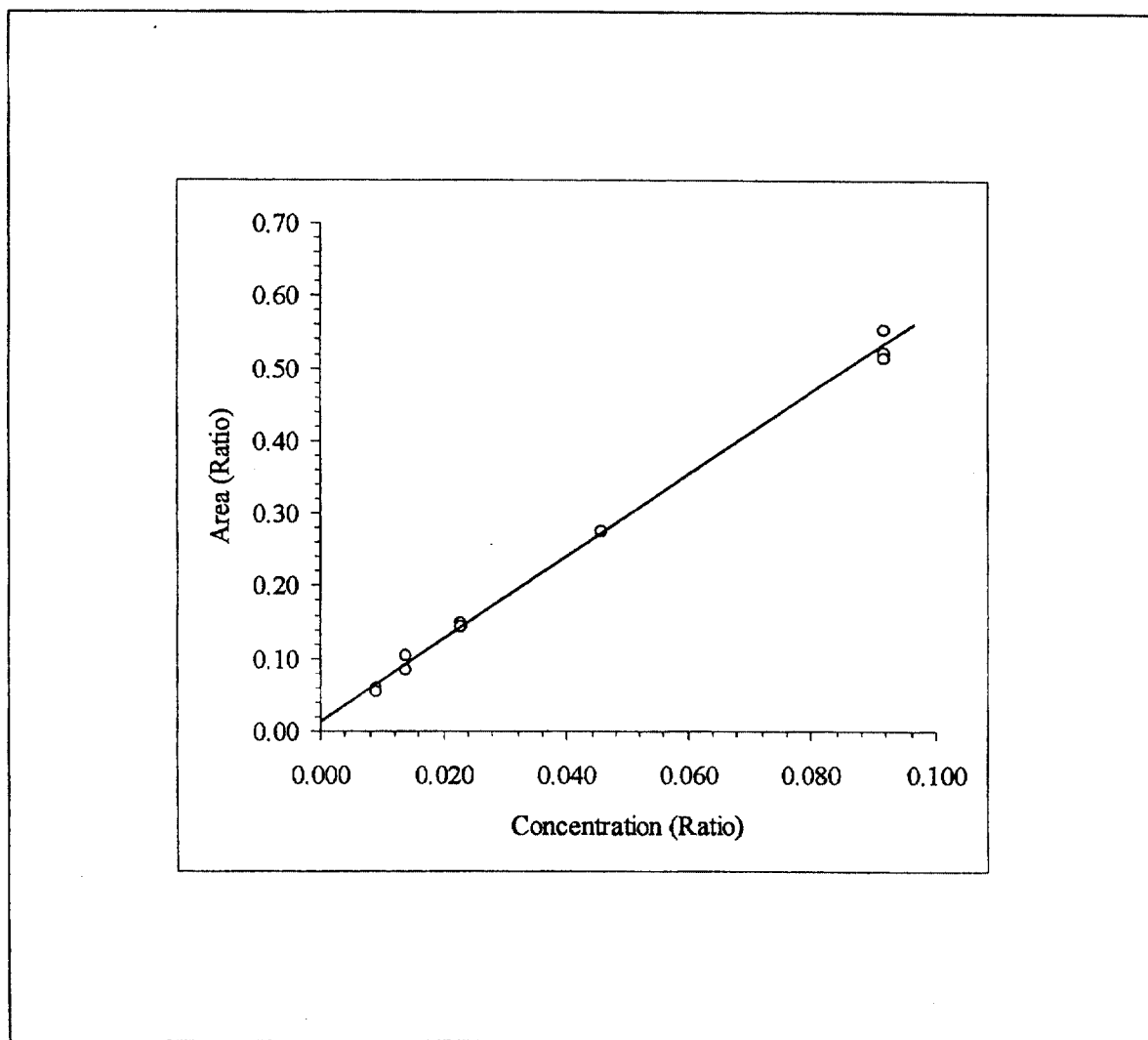


Figure 2. A typical calibration curve for PFOS. Slope = 5.76026; Intercept = 0.00940; $r = 0.9974$. Curve is weighted ($1/x$).

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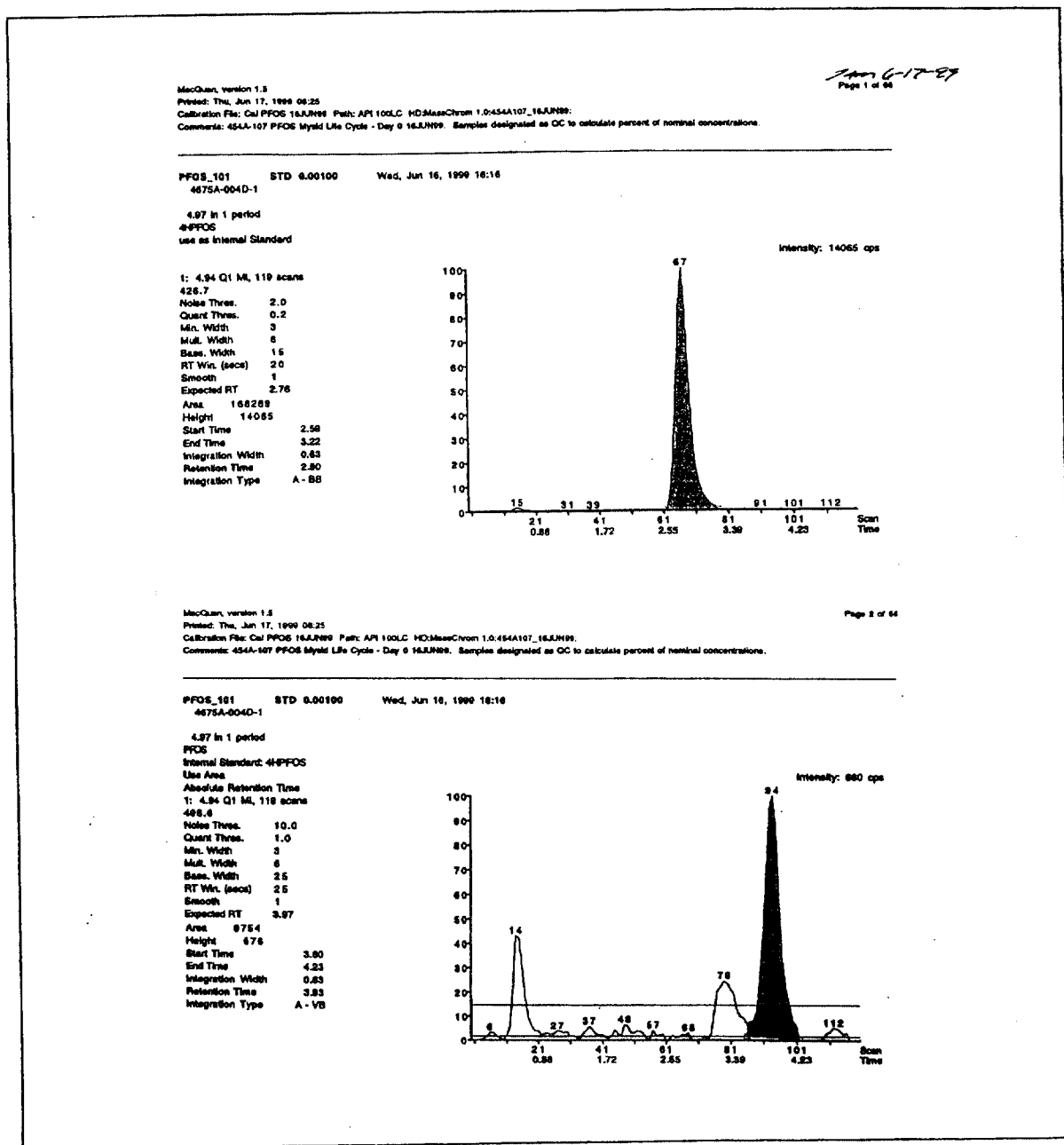


Figure 3. A representative ion chromatogram of a low-level (0.000915 mg a.i./L) PFOS standard.

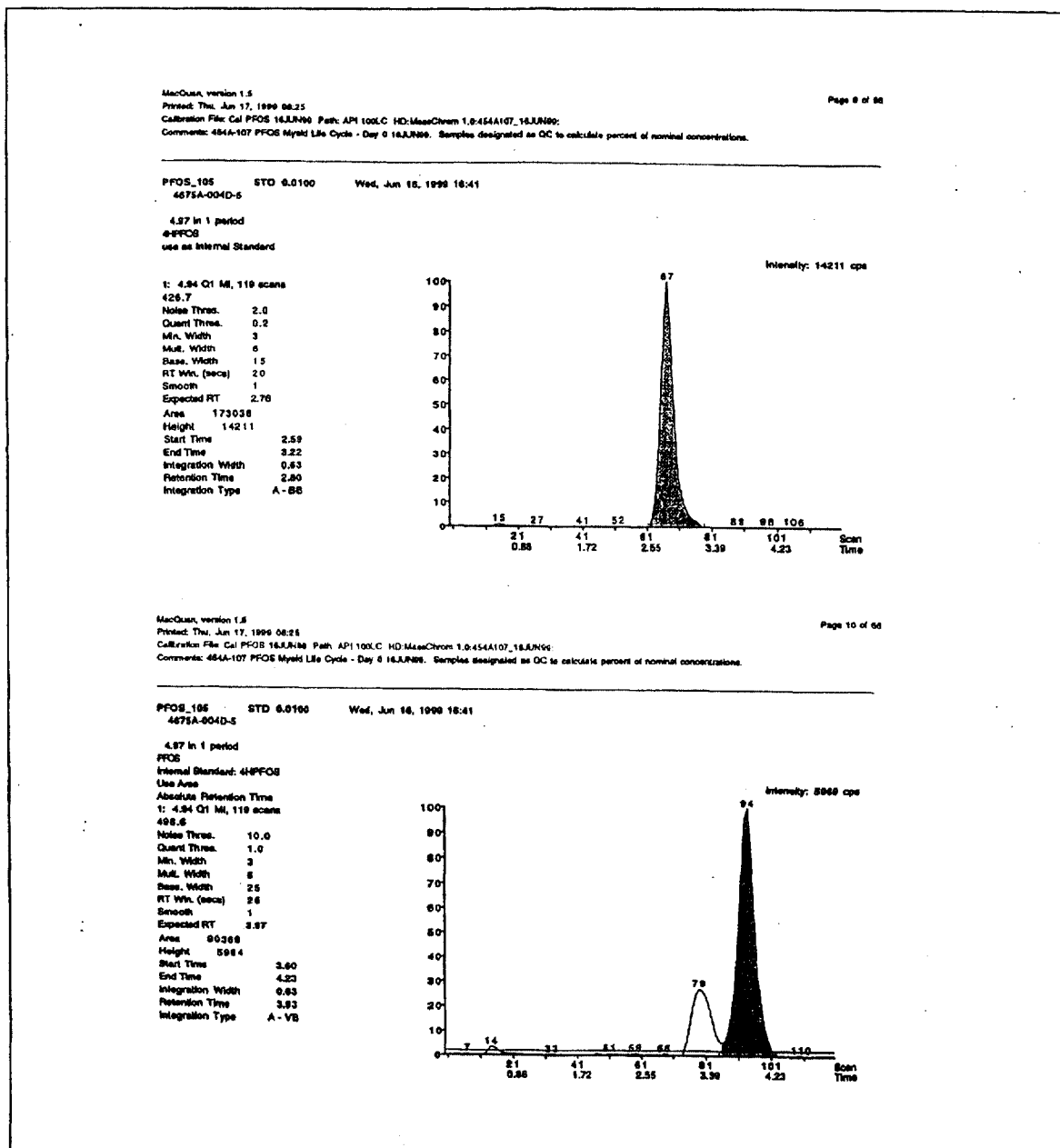


Figure 4. A representative ion chromatogram of a high-level (0.00915 mg a.i./L) PFOS standard.

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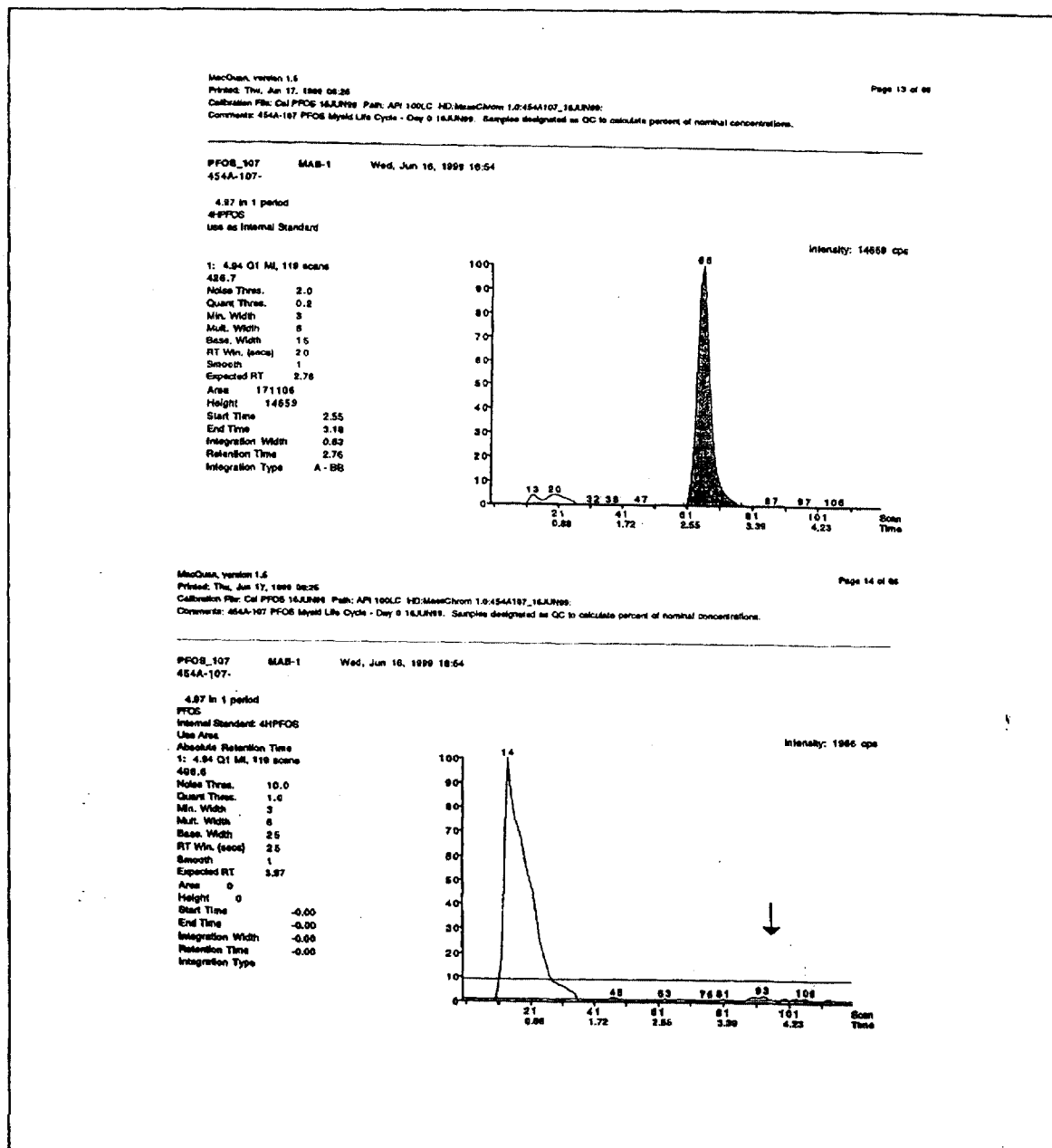


Figure 5. A representative ion chromatogram of a matrix blank sample (454A-107-MAB-1). The arrow indicates the retention time of PFOS.

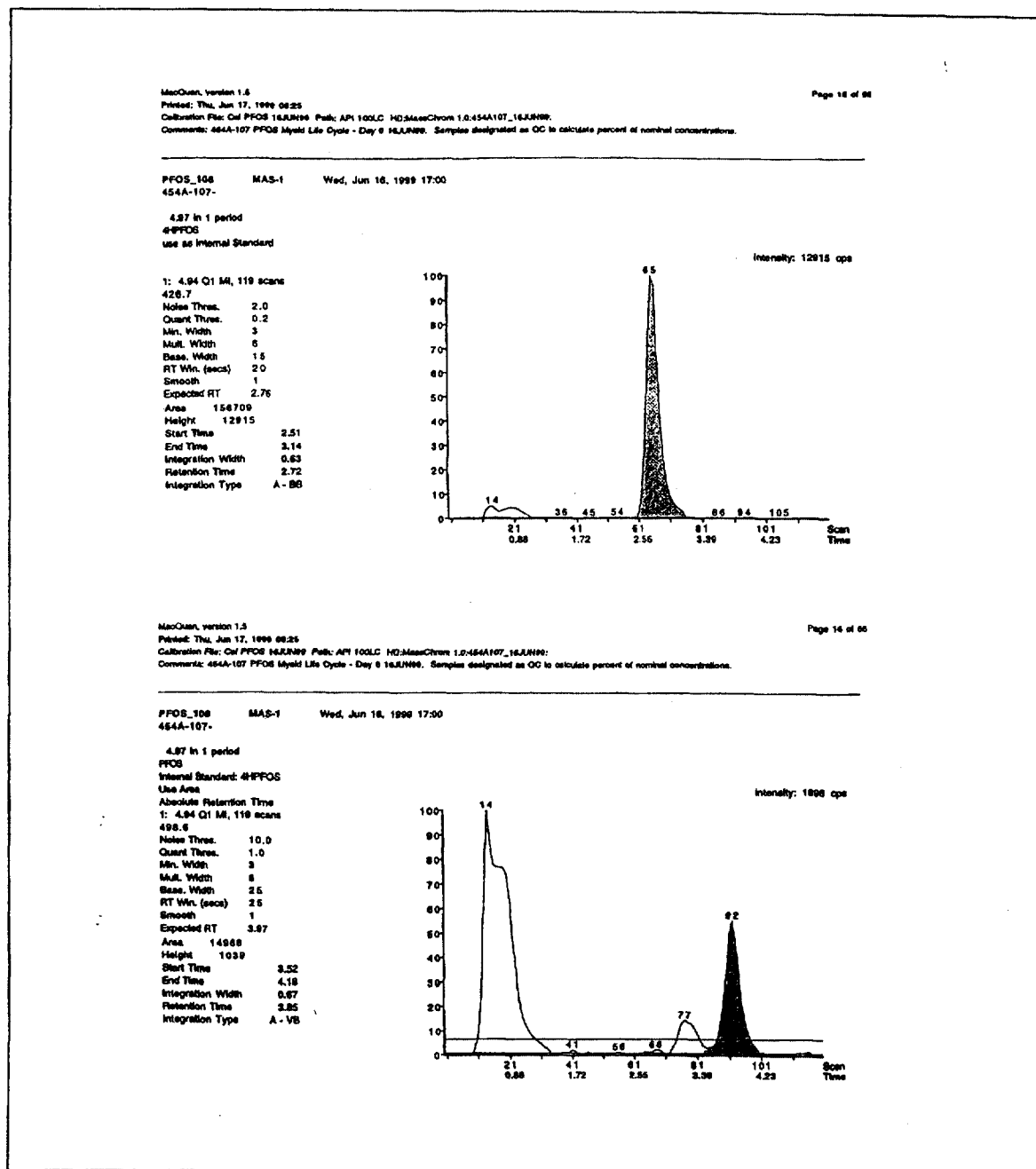


Figure 6. A representative ion chromatogram of a matrix fortification sample (454A-107-MAS-1).

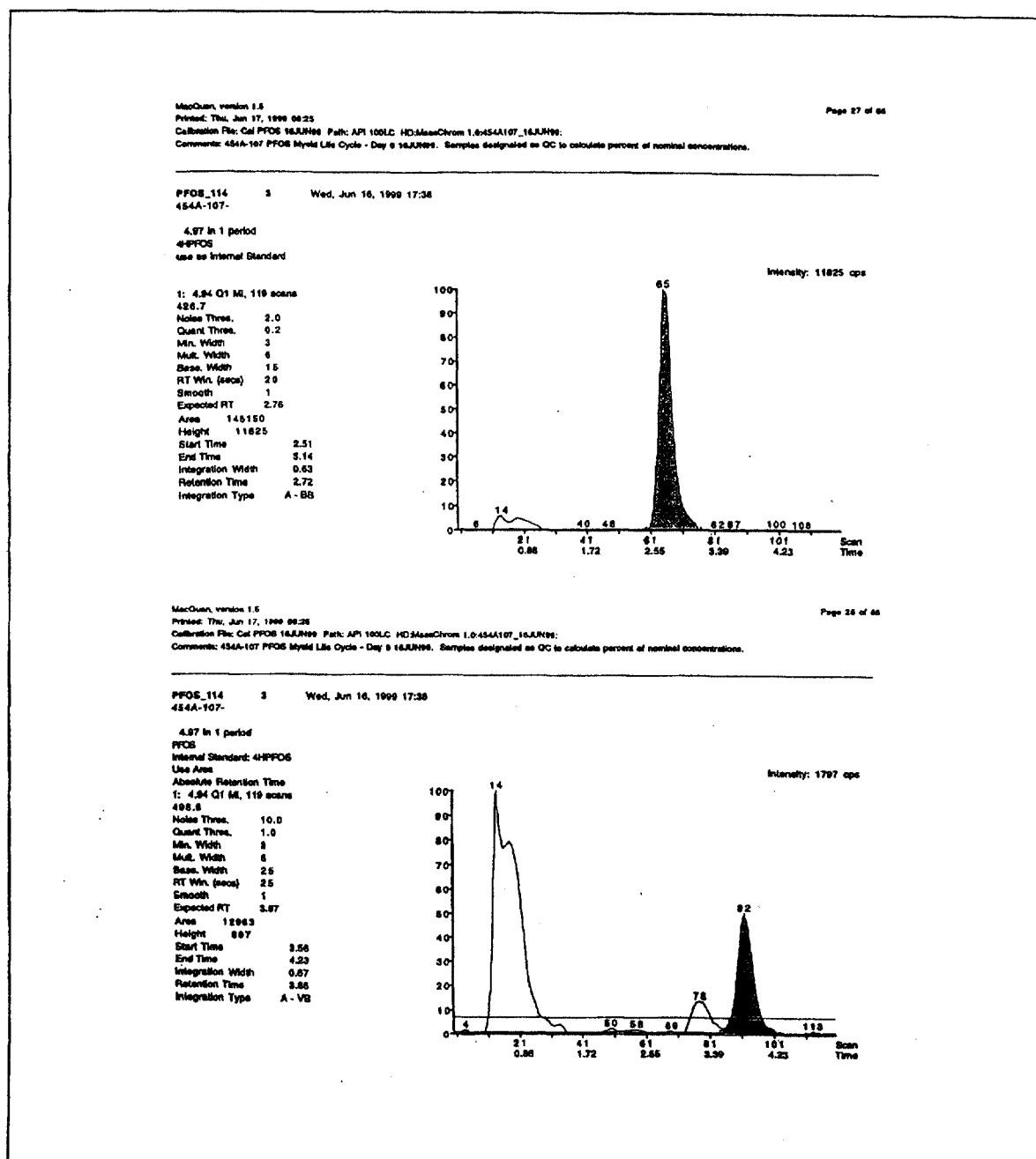


Figure 7. A representative ion chromatogram of a test sample (454A-107-3).

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APPENDIX IV

Mysid Reproduction

Sponsor:		3M Corporation			
Test Substance:		PFOS			
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water:		Filtered Saltwater			
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Compartment	Number of Reproductive Days ¹	Number of Young Produced	Number of Young/Reproductive Day
Negative Control	A	1	14	3	0.257
		2	14	2	
		3	14	6	
		4	14	5	
		5	14	2	
	B	1	14	4	0.264
		2	14	8	
		3	14	2	
		4	11	0	
	C	1	14	6	0.286
		2	14	3	
		3	14	4	
		4	14	5	
		5	14	2	
	D	1	14	9	0.452
		2	14	6	
		3	14	4	
0.057	A	1	14	3	0.283
		2	14	6	
		3	14	3	
		4	14	5	
		5	4	0	
	B	1	14	2	0.200
		2	14	3	
		3	14	5	
		4	14	0	
		5	14	4	
	C	1	14	3	0.186
		2	14	3	
		3	14	3	
		4	14	0	
		5	14	4	
	D	1	14	2	0.375
		2	14	10	
		3	14	7	
		4	14	2	

¹ The number of reproductive days is the number of days that the female was alive from first brood release to the end of the test.

APPENDIX IV (Continued)

Mysid Reproduction

Sponsor:		3M Corporation			
Test Substance:		PFOS			
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water:		Filtered Saltwater			
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Compartment	Number of Reproductive Days ¹	Number of Young Produced	Number of Young/Reproductive Day
0.12	A	1	14	6	0.300
		2	14	0	
		3	14	8	
		4	14	6	
		5	14	1	
	B	1	14	7	0.478
		2	14	8	
		3	14	5	
		4	4	2	
	C	1	14	7	0.407
		2	14	9	
		3	14	4	
		4	12	2	
	D	1	14	9	0.257
		2	14	2	
		3	14	0	
		4	14	5	
		5	14	2	
0.25	A	1	14	2	0.271
		2	14	4	
		3	14	2	
		4	14	6	
		5	14	5	
	B	1	14	4	0.214
		2	14	1	
		3	14	7	
		4	14	0	
	C	1	14	2	0.344
		2	5	2	
		3	14	7	
		4	14	6	
		5	14	4	
	D	1	14	1	0.179
		2	14	0	
		3	14	3	
		4	14	6	
		5			

¹ The number of reproductive days is the number of days that the female was alive from first brood release to the end of the test.

APPENDIX IV (Continued)

Mysid Reproduction

Sponsor:		3M Corporation			
Test Substance:		PFOS			
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water:		Filtered Saltwater			
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Compartment	Number of Reproductive Days ¹	Number of Young Produced	Number of Young/Reproductive Day
0.55	A	1	14	0	0.0556
		2	14	3	
		3	12	0	
		4	14	0	
	B	1	14	0	0.107
		2	14	0	
		3	14	2	
		4	14	4	
	C	1	14	0	0.0429
		2	14	0	
		3	14	0	
		4	14	3	
		5	14	0	
	D	1	14	0	0.0179
		2	14	0	
		3	14	0	
		4	14	1	
1.3	A	1	3	0	0.000
		2	11	0	
		3	8	0	
	B	1	14	0	0.000
	C	--	--	--	--
	D	1	11	0	0.000

¹ The number of reproductive days is the number of days that the female was alive from pairing to the end of the test.

APPENDIX V

Mysid Total Length (mm)

Mysid Number	Negative Control				0.57 mg a.i./L				0.12 mg a.i./L			
	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D
1	6.80	6.20	6.50	6.55	6.15	6.35	6.05	6.30	6.25	6.75	6.90	6.30
2	6.85	6.65	6.85	7.10	6.30	6.20	6.80	6.45	6.70	6.60	6.70	6.75
3	6.30	6.55	6.20	6.35	6.35	6.15	6.55	6.40	6.40	6.55	6.45	6.40
4	6.45	6.95	6.60	6.10	6.15	6.70	6.70	6.90	6.55	6.80	7.15	6.40
5	6.30	5.90	6.25	6.40	6.55	6.45	6.45	6.55	6.60	6.45	6.35	6.10
6	6.75	6.10	6.40	6.45	6.90	6.60	6.65	6.45	7.10	6.90	6.60	6.15
7	6.15	6.00	6.15	6.30	6.20	6.30	6.50	5.90	6.35	6.50	6.20	6.30
8	6.65	-	6.30	6.55	6.65	6.80	6.70	6.35	6.40	-	-	6.80
9	6.30	-	6.70	-	6.25	6.30	6.30	5.95	6.25	-	-	6.40
10	6.50	-	6.65	-	6.40	6.40	6.50	6.35	7.00	-	-	6.55
11	5.85	-	-	-	6.15	6.50	-	-	6.55	-	-	-
12	-	-	-	-	6.50	6.65	-	-	6.40	-	-	-
Mean	6.45	6.34	6.46	6.48	6.38	6.45	6.52	6.36	6.55	6.65	6.62	6.42

APPENDIX V (Continued)

Mysid Total Length (mm)

Mysid Number	0.25 mg a.i./L				0.55 mg a.i./L				1.3 mg a.i./L			
	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D
1	6.55	6.25	5.95	6.20	6.05	6.05	6.20	6.20	5.85	5.75	-	5.65
2	6.40	6.40	6.35	6.45	5.85	6.25	6.20	6.45	6.05	6.25	-	-
3	6.35	6.30	6.80	6.50	6.10	6.35	5.85	5.95	5.90	5.85	-	-
4	6.45	6.15	6.10	6.35	5.90	6.20	6.00	6.10	-	6.05	-	-
5	6.50	6.45	6.75	6.75	5.80	6.00	6.10	6.25	-	-	-	-
6	6.25	6.90	6.20	6.55	6.35	6.20	6.35	6.40	-	-	-	-
7	6.55	6.35	6.40	6.05	6.30	6.15	6.25	6.10	-	-	-	-
8	6.70	6.20	6.35	6.80	-	6.60	6.40	6.45	-	-	-	-
9	6.45	-	6.35	5.75	-	5.05	6.25	-	-	-	-	-
10	6.60	-	-	-	-	6.25	6.00	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
Mean	6.48	6.38	6.36	6.38	6.05	6.11	6.16	6.24	5.93	5.98	-	5.65

APPENDIX VI

Mysid Dry Weight (mg)

Sponsor:		3M Corporation											
Test Substance:		PFOS											
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>											
Dilution Water:		Filtered Saltwater											
Mysid Number		Negative Control				0.057 mg a.i./L				0.12 mg a.i./L			
		Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D
1		0.51	0.57	0.49	0.68	0.53	0.65	0.45	0.59	0.52	0.87	0.78	0.54
2		0.70	0.77	0.78	0.86	0.62	0.78	0.50	0.51	0.71	0.62	0.52	0.68
3		0.52	0.70	0.60	0.54	0.58	0.55	0.60	0.46	0.65	0.55	0.56	0.52
4		0.64	0.86	0.57	0.59	0.64	0.69	0.56	0.80	0.70	0.68	0.88	0.67
5		0.56	0.61	0.51	0.53	0.82	0.47	0.63	0.37	0.72	0.64	0.55	0.46
6		0.75	0.75	0.72	0.67	0.79	0.84	0.59	0.52	0.80	0.71	0.72	0.55
7		0.44	0.68	0.50	0.60	0.51	0.52	0.54	0.46	0.54	0.58	0.50	0.64
8		0.79	--	0.67	0.62	0.66	0.73	0.71	0.67	0.58	--	--	0.71
9		0.58	--	0.52	--	0.49	0.61	0.49	0.49	0.59	--	--	0.74
10		0.69	--	0.60	--	0.57	0.72	0.55	0.58	0.83	--	--	0.56
11		0.41	--	--	--	0.50	0.66	--	--	0.49	--	--	--
12		--	--	--	--	0.68	0.58	--	--	0.63	--	--	--
Mean		0.599	0.706	0.596	0.636	0.616	0.627	0.590	0.565	0.647	0.664	0.644	0.607

APPENDIX VI (Continued)

Mysid Dry Weight (mg)

Sponsor:		3M Corporation											
Test Substance:		PROS											
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>											
Dilution Water:		Filtered Saltwater											
Mysid Number	0.25 mg a.i./L				0.55 mg a.i./L				1.3 mg a.i./L				
	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	
1	0.72	0.45	0.30	0.61	0.62	0.50	0.57	0.54	0.36	0.40	-	0.39	
2	0.60	0.68	0.62	0.67	0.51	0.48	0.42	0.66	0.32	0.37	-	-	
3	0.54	0.62	0.86	0.39	0.54	0.67	0.39	0.52	0.44	0.43	-	-	
4	0.55	0.33	0.45	0.53	0.45	0.62	0.61	0.88	-	0.51	-	-	
5	0.63	0.58	0.71	0.84	0.43	0.37	0.51	0.55	-	-	-	-	
6	0.48	0.79	0.55	0.73	0.71	0.61	0.60	0.60	-	-	-	-	
7	0.79	0.60	0.51	0.52	0.63	0.54	0.81	0.49	-	-	-	-	
8	0.71	0.56	0.67	0.80	-	0.59	0.65	0.52	-	-	-	-	
9	0.67	-	0.56	0.46	-	0.36	0.48	-	-	-	-	-	
10	0.75	-	-	-	-	0.64	0.39	-	-	-	-	-	
11	-	-	-	-	-	-	-	-	-	-	-	-	
Mean	0.644	0.601	0.603	0.639	0.536	0.558	0.563	0.570	0.440	0.478	-	0.39	

APPENDIX VII

Changes to Protocol

This study was conducted in accordance with the approved Protocol with the following changes:

1. The proposed experimental start and termination dates were amended to the protocol.
 2. Analysis of feed for PFOS was deleted by amendment.
 3. The proportion of water split to each replicate was not checked at the end of the test.
 4. Temperature was not measured in the B replicate of the 2.7 mg a.i./L treatment group on Day 14 of the test.
 5. Nominal test concentrations were 0.086, 0.17, 0.34, 0.69, 1.4 and 2.7 mg a.i./L.
-

APPENDIX VIII

Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
2. Willard B. Nixon, Ph.D., Manager, Analytical Chemistry
3. Jon A. MacGregor, Scientist
4. Mark A. Mank, Laboratory Supervisor
5. Kurt R. Drottar, Senior Biologist

- 1 -

PFOS:
A FLOW -THROUGH LIFE-CYCLE TOXICITY TEST WITH
THE SALTWATER MYSID (*Mysidopsis bahia*)

ADDENDUM

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-107
3M LAB REQUEST NO. U2723

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.1350

This study was reported using a test substance purity of 90.49%. The test substance characterization was revised on 9/7/00 and the purity was determined to be 86.9%. The Sponsor requested that the results be revised based on the most recent purity analyses. The results previously presented were simply corrected by the ratio of the two purities or a factor of 0.9603. No attempt was made to recalculate analytical standard concentrations or measured concentrations from original raw data. As such, minor errors due to rounding may be present. Attached is the revised summary page from the final report and the most recent certificate of analysis.

REVISED STUDY SUMMARY BASED ON A PURITY OF 86.9%

SPONSOR:	3M Corporation
SPONSOR'S REPRESENTATIVE:	Susan A. Beach
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	454A-107
TEST SUBSTANCE:	PFOS (Perfluorooctane Sulfonic Acid Potassium Salt)
STUDY:	PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (<i>Mysidopsis bahia</i>)
NOMINAL TEST CONCENTRATIONS:	Negative Control, 0.083, 0.16, 0.33, 0.66, 1.3 and 2.6 mg a.i./L
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control, 0.055, 0.12, 0.24, 0.53, 1.2 and 2.5 mg a.i./L
TEST DATES:	Experimental Start (OECD) - May 26, 1999 Experimental Start (EPA) - June 16, 1999 Biological Termination - July 25, 1999 Experimental Termination - July 25, 1999
LENGTH OF FIRST- GENERATION EXPOSURE:	35 Days

TEST ORGANISM:	Saltwater Mysid (<i>Mysidopsis bahia</i>)
SOURCE OF TEST ORGANISMS:	Wildlife International, Ltd. Cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	Juveniles <24 hours old

NOEC:	0.24 mg a.i./L
LOEC:	0.53 mg a.i./L
MATC:	0.36 mg a.i./L

CERTIFICATE OF ANALYSIS

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018A

3M Product: PFOS, Lot 217

Reference #: SD-018

Purity: 86.9%

Test Name	Specifications	Result
Purity ¹		86.9%
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium ² 5. Nickel 6. Iron 7. Manganese		1. 0.005 wt./wt.% 2. 0.001 wt./wt.% 3. 1.439 wt./wt.% 4. 6.849 wt./wt.% 5. <0.001 wt./wt.% 6. 0.005 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.93 wt./wt.%
Total % Impurity (LC/MS)		8.41 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.33 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable ³
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate ⁴		1. <0.015 wt./wt.% 2. 0.59 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.76 wt./wt.%
Organic Acids ⁵ (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. 0.10 wt./wt.% 4. 0.28 wt./wt.%
Elemental Analysis ⁶ : 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	1. 12.48 wt./wt.% 2. 0.244 wt./wt.% 3. 1.74 wt./wt.% 4. 8.84 wt./wt.% 5. 54.1 wt./wt.%

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen \square -10°C

Re-assessment Date: 08/31/01

¹Purity = 100% - (sum of metal impurities, 1.45% + LC/MS impurities, 8.41% + Inorganic Fluoride, 0.59% + NMR impurities, 1.93% + organic acid impurities, 0.38% + POAA, 0.33%)

Total impurity from all tests = 13.09%

Purity = 100% - 13.09% = 86.9%

²Potassium is expected in this salt form and is therefore not considered an impurity.

³Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

⁴Sulfur in the sample appears to be converted to SO₄ and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO₄ is not considered an impurity.

⁵ TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

⁶Theoretical value calculations based on the empirical formula, C₈F₁₇SO₃K⁺ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

INTERIM CERTIFICATE OF ANALYSIS
Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.22
C5	1.33
C6	4.72
C7	1.14
Total	8.41

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: _____

David S. Bell
Scientist, Centre Analytical Laboratories

Date

Reviewed By: _____

John Flaherty
Laboratory Manager, Centre Analytical Laboratories

Date